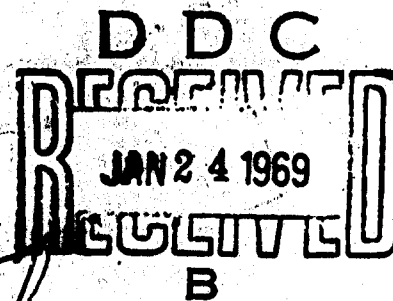


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## IMMUNOGENESIS IN ANTI-CHOLERA VACCINATION WITH THE USUAL AND WITH SHORTENED INTERVALS BETWEEN INOCULATIONS (PRELIMINARY COMMUNICATION)

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IN RECENT years a number of papers have been published by Soviet authors dealing with the rate of development of immunity as a result of vaccination. Pletsityi, Alymov, Aver'ianova, Labinskaia and Aksenova quote experimental data which indicate the possibility of developing immunity in an animal with extremely short intervals between inoculation, of hours or even minutes and seconds.

Proceeding from the laws of physiology the authors consider debatable the accepted hypothesis of the slow development of immunity in response to the introduction of various antigens. They showed that there is probably no difference in that respect between antigens and other stimuli in their effect on complex organisms. In their experiments with tetanus toxoid, as an example these authors showed that on the production of antitoxic immunity the reactivity of the animal changes very rapidly under the influence of introduced antigen, both by primary vaccination and by revaccination.

The problem of creating specific immunity in a reduced time period has also, in our opinion, a great practical as well as theoretical importance, especially bearing in mind a number of infections which tend to spread rapidly by epidemic. As it is known, a complete course of vaccination with the usual intervals takes 15-20 days, but using shortened intervals it should be possible to complete it in several days or even hours and minutes.

The present communication deals with features of development of immunity against cholera by such a shortened vaccination scheme.

In order to investigate the rate of production of serum agglutinins and bactericidal activity in conditions of shortened intervals a group of rabbits was immunized. A survival test was carried out with mice under the same conditions. We used a heated monovalent vaccine prepared from *Vibrio cholerae* strain no. 1488, Inaba type. The vaccine was administered 3 times, according to the following scheme: with usual 7 day intervals (first group), with 24 hr intervals (second group), and with 5 hr intervals between the separate inoculations (third group). Animals in the second group received the vaccine daily during the last three days of the immunization cycle of the first group. Animals in the third group received the vaccine three times on the day of the final immunization of the first group (there were 4 rabbits in each group).

The rabbits were vaccinated intramuscularly in doses of 2,000 and 4,000 million active doses and the mice subcutaneously in doses of 250, 500, and 500 million. The rate of development of various immunity characteristics in the rabbits was studied for 70 days after the end of vaccination. The results of agglutinin titrations given in Fig. 1 show that the highest titres were obtained in the group with 7 day intervals between injections, followed by the sera of animals immunized at daily intervals, and the last place was occupied by the sera of animals immunized at 5 hr intervals. In the groups with shortened intervals, maximum titres were recorded much later than in the first group. The time of fall of agglutinin titre was approximately the same in all three groups.

Therefore, were one to judge the degree of immunization by agglutinins, it appears to be greatest in animals inoculated at the usual 7 day intervals, somewhat less effective

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with daily, and least effective with 5 hr intervals between the separate inoculations. Consequently, on reducing the times between the successive antigenic stimuli the immune response appeared more slowly and to a lesser degree.

A different picture was obtained on investigating the bactericidal properties of the same sera towards the same strain of *V. cholerae*. This protective property plays a very important role in cholera vaccination, and possibly reflects to a large extent the real degree of immunity.

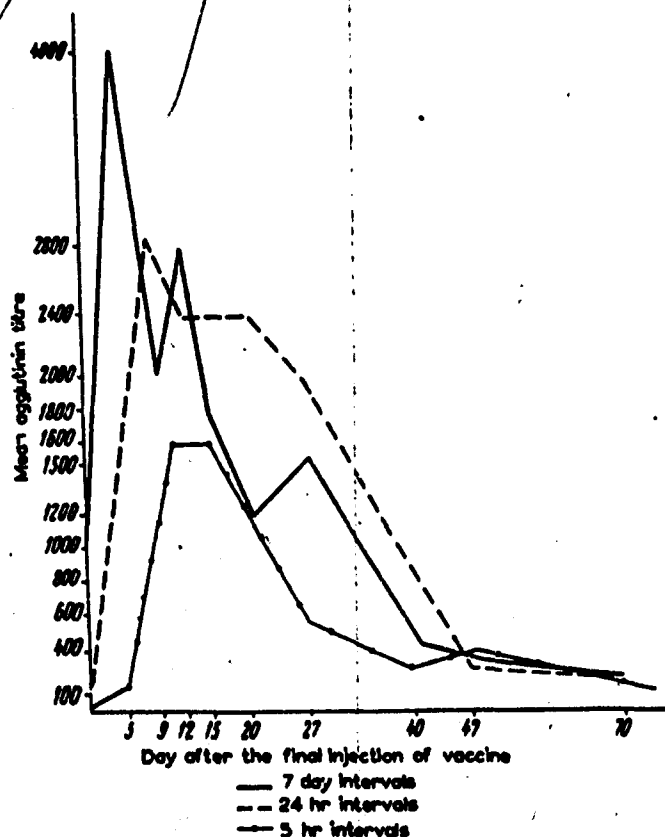


FIG. 1. Course of agglutinin titres of rabbit sera with vaccination at different intervals.

Bactericidal activity was investigated by the following method: 20–24 hr after taking blood the individual sera were diluted with saline from 1 : 2 to 1 : 128 and to each dilution one drop of a 24 hr agar culture of *V. cholerae* containing 10 million organisms was added; after incubating for 4 hr at 37°C one loopful of the mixture (diameter 4 mm) was plated on solid Smirnov medium and after 24 hr growth at 37° the number of colonies was counted.

The bactericidal effect of the sera was expressed as the difference between the number of colonies in the control (saline + 1 drop of culture) tube and in the experimental one.

Variations in the bactericidal effect of the sera are shown in Fig. 2. First of all it ought to be mentioned that after vaccination the bactericidal activity of sera in all groups rose sharply. The largest increase over the initial figure was obtained in the group with 7 day intervals, and a somewhat smaller and almost identical one in the other two groups. The further course of the curves of bactericidal activity within the range of the observation period is characterized by several special features: in the group with 7 day

and to a lesser extent with daily intervals a fluctuation in bactericidal activity was observed, whereas in the group with the 5 hr intervals the results were constant.

On comparing the agglutination curves with the curves of bactericidal activity of sera of the same animals the well-known parallelism during the first post-vaccination period and the divergence towards the end of the observation period can be seen. Although

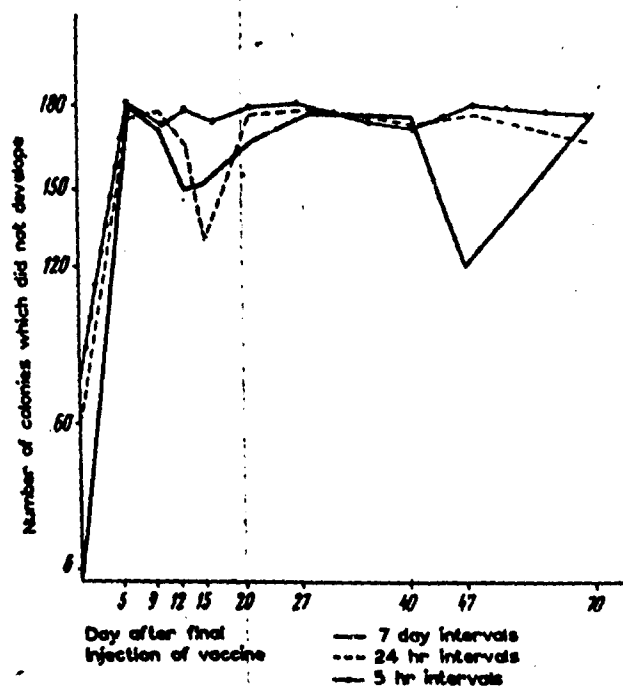


FIG. 2. Course of bactericidal activity of rabbit sera with vaccination at different intervals.

towards the seventieth day agglutinin titres fell almost to the initial level the bactericidal activity remained at a high level in all groups. We did not observe any substantial differences between the individual groups in this respect.

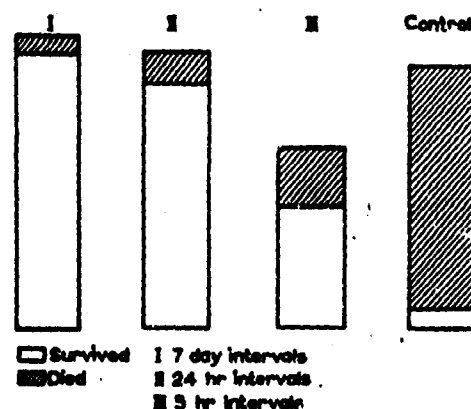


FIG. 3. Investigation of active immunity of mice vaccinated at different intervals.

In addition to the rate of development of these antibodies we used a more reliable criterion of progress of immunity, the investigation of survival, carried out in a small

group of mice on the twelfth day after the end of vaccination. Immunized mice were infected with one lethal dose of cholera culture strain no. 1488. We used a suspension in peptone water of a 24 hr agar culture. One lethal dose is equal to 125–250 million bacterial cells per ml. During the vaccination period some deaths of mice were observed: in the group with 5 hr intervals 15 of the 20 vaccinated mice remained, with daily intervals 17 out of 20, and with 7 day intervals 18 out of 20.

As can be seen from Fig. 3, where the results of the above test are given, a sufficiently high survival was obtained in all three groups of animals, namely, in the first group 17 out of 18, in the second 15 out of 17, and in the third 11 out of 15; in the control group only one of the 16 mice survived.

Therefore, our results show that despite the sharp reduction of intervals between inoculations, in comparison with those accepted in practice, a sufficiently pronounced immunity developed. In order to verify our results it is necessary to carry out further work, in course of which different schemes of so-called rapid immunization should be used.

### CONCLUSIONS

(1) The intensity of development of agglutinins in animals vaccinated against cholera with different intervals between inoculations was directly proportional to the length of the interval.

(2) The bactericidal activity of these sera rose independently of the duration of intervals; it lasted longest and was most constant in the case of rabbits immunized with 5 hr intervals between injections.

(3) A direct relationship was revealed between the bactericidal activity and agglutinin titre of the same sera during the first post-vaccination period, and an inverse one towards the end. Therefore agglutinin titre is an inadequate criterion of degree of immunity.

(4) On investigating the active immunity of mice with different intervals nearly identical results were obtained, indicating that a sufficient degree of immunity developed on vaccinating animals by any of the methods which we used. ( ) ←

*Translated by J. ROBYN*

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